1028-170 Synthetic Small Molecule Inhibitor of P, E And L Selectin Given Just Prior to Reperfusion Produces Infarct Salvage in a Two Hour Canine Coronary Occlusion/Reperfusion Model

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Background: Reperfusion in the setting of acute myocardial infarction may be associated with increased mortality in the first 24 hours of hospital admission, secondary to reperfusion injury. We tested the hypothesis that TBC 1269, a synthetic small molecule inhibitor of P, E, and L selectin, attenuates neutrophil activation and results in infarct salvage in a canine infarction-reperfusion model.

Methods: Twenty-two open-chest dogs were randomized to receive TBC 1269, 35 mg/kg intravenous bolus followed by infusion, or placebo, after 105 minutes of left anterior descending coronary artery occlusion (15 minutes prior to reperfusion). The infusion of TBC 1269 continued throughout the reperfusion period of 4 hours. Hemodynamic assessment, regional myocardial blood flow determination with radioactive microspheres, myocardial leukocyte infiltration by myeloperoxidase assay, and estimation of infarct size using triphenyl tetrazolium chloride staining were performed.

Results: Infarct size as a percentage of zone at risk was significantly reduced ($P < 0.05$, analysis of co-variance) in the TBC 1269 arm versus placebo (mean 38 ± 16 vs 55 ± 11% respectively) independent of collateral blood flow. Myeloperoxidase activity was reduced in the infarct and ischemic zones as compared with placebo. Hemodynamic parameters did not differ significantly between the two groups.

Conclusion: TBC 1269, a specific small molecule antagonist of P, E and L selectin, administered as bolus prior to reperfusion and continuous infusion throughout reperfusion results in reduced neutrophil activation and infarct salvage without adverse hemodynamic events.

1028-171 Incidence of Conduction Disturbances Complicating Acute Myocardial Infarction With Amiodarone at Moderate and High Doses (Observations from GEMICA Study)

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No data exists regarding incidence of conduction disturbances (CD) with and without IV amiodarone (A), a class III antiarrhythmic drug, in acute myocardial infarction. This substudy is an independent analysis from the GEMICA study, a multicenter randomized, double-blind, placebo controlled study designed to evaluate the effects of early intravenous and oral administration of A vs placebo (P) added to the conventional therapy in AMI. A was administered initially as follows: i.v. 1350 mg over 48 h + oral 1200 mg qd. × 4, 400 mg qd × 3 months and 200 mg qd × 3 months. After de inclusion of 516 patients (group I) the above mentioned protocol was shifted to i.v. 600 mg over 48 h + oral 800 mg qd. × 2 and 400 mg qd × 3 months and 200 mg qd × 3 months respectively (group II). From March 1994 to August 1995, 1073 patients with AMI were enrolled. During hospitalization, 32 out of 534 P developed new CD (6%) and 46 from 539 A (8.5%) [p = NS]. In group I, incidence of CD was 29 in A vs. 12 in P [$p < 0.05$] and in group II 17 vs. 20 respectively [p = NS]. The bundle branch block incidence in group I was 13 with A vs. 2 with P [$p < 0.01$] and NS in group II, and the presence of 2nd. and 3rd. AV block was not significative (18 in 534 P group vs. 22 in 539 A group, irrespectively of the dose). Bradicardia required temporary pacing in 12 A and 5 P pts. from group I and 5 and 8 in group II [pNS]. Only 1 patient required definitive pacing, from control group. In conclusion A does not enhance CD significantly in moderate doses in the presence of AMI.

1028-172 Mechanistic Spectrum of Adenosine-Sensitive Atrial Tachycardia

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Adenosine's (Ado) electrophysiologic effects on supraventricular tissue are mediated through either activation of I_{KAdo} or antagonism of cAMP-stimulated currents. We have previously shown that Ado suppresses conduction in decremental atrial tissue, transiently suppresses automatic atrial tachycardia (AT), and has no effect on reentrant AT. To further elaborate Ado's effects on AT, we assessed the effects of Ado on 26 consecutive ATs (mean cycle length 366 ± 122 ms), excluding typical atrial flutter, in 25 pts (age 51 ± 21, 14 F). Thirteen ATs terminated with Ado and could be classified as follows:

Pts	Origin	Induction	Termination	Mechanism
6	high lat. RA	prog-stim	Ado (+)	sinus node reentry
2	low lat. RA	prog-stim	Ado (+)	cristal reentry
1	septal RA	prog-stim	Ado (+)	macroreentry + decremental conduction†
1	mid lat. RA	iso + prog-stim verapamil (−)	Ado (+)	catecholamine facilitated reentry
3*	lat. RA septal RA RA append.	iso + prog-stim verapamil (+) valsalva (+)	Ado (+)	cAMP-mediated triggered activity

*Each of these pts had repetitive monomorphic AT. In one pt, delayed after depolarizations were demonstrated with a monophasic action potential recording and were abolished by Ado and verapamil. † Ado terminated AT in a decremental zone of slow conduction.

In 6 pts with intraatrial reentrant AT complicating atrial surgery for congenital or acquired heart disease, Ado had no effect on AT. These diverse responses of AT to Ado are all explained by the mechanism specific effects of Ado on I_{KAdo} and antagonism of cAMP.

1029 Cardiovascular Molecular Biology: Myocardial Function and Mass

Tuesday, March 18, 1997, 3:00 p.m.–5:00 p.m.
Anaheim Convention Center, Hall E
Presentation Hour: 3:00 p.m.–4:00 p.m.

1029-161 Immunolocalization of Activated Epsilon Protein Kinase C in Isolated Adult Cardiomyocytes

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Immunofluorescence studies in neonatal cultured myocytes have recently demonstrated that activation of epsilon protein kinase C (ε-PKC) may play a role in mediating the protective effects of ischemic preconditioning against anoxia-reoxygenation injury. Because the more clinically relevant situation of ischemia-reperfusion can only be replicated in whole hearts, it would prove valuable to assess PKC activation in adult myocytes, isolated from whole hearts. Therefore, immunolocalization of ε-PKC was performed in myocytes isolated by collagenase digestion of hearts from adult guinea pigs. Unstimulated myocytes and myocytes exposed to the PKC activator, phorbol 12-myristate 13-acetate (PMA; 100 nM for 15 min) were studied. Myocytes were fixed in cold methanol:aceton (1:1) solution. Fixed myocytes were incubated with a primary antibody to ε-PKC, followed by a fluorescein-conjugated secondary antibody. In unstimulated myocytes, fluorescence was primarily in the nucleus. Following activation with PMA, fluorescence was increased at cross-striated structures (likely myofilaments), and decreased in the nucleus, suggesting translocation (activation) of ε-PKC. This pattern of ε-PKC translocation is analogous to that seen in neonatal myocytes following PMA treatment or ischemic preconditioning. This is the first description of the immunofluorescence characteristics of activation of ε-PKC in myocytes isolated from adult hearts. This technique may be useful in defining the role of PKC isozyme activation during the clinically relevant situation of ischemia-reperfusion injury in myocytes isolated from adult hearts.

1029-162 Naturally-Occurring Silent Sequence Polymorphisms in the Human β Myosin Heavy Chain Gene

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Over 40 mutations in the β myosin heavy chain (MHC) gene have been reported to be associated with familial hypertrophic cardiomyopathy (FHC). While some of these mutations would be predicted to severely alter myosin function, others would be predicted to be mild, suggesting that the cardiac MHC gene may not be able to tolerate many changes. In order to determine how polymorphic the β MHC gene is in normal individuals, we studied the human β MHC genes from 25 unrelated individuals (50 alleles). Their ethnic backgrounds were: 22 white, 2 black and one hispanic. Samples were screened for polymorphisms either by reverse transcriptase-polymerase chain reaction (RT-PCR) of RNA from the left ventricles followed by cloning and sequencing or by single strand DNA conformation polymorphism (SSCP). We screened exons 2–14 and 33–40 (corresponding to a little less than half of the total exons) and found six nucleic acid polymorphisms in exons 2–14. However, none of them resulted in an amino acid change. Only one polymorphism was found in the 3′ untranslated region. Thus, the human

β MHC gene is extremely highly conserved at the amino acid level in both head and rod regions and even neutral polymorphisms appear to be rare.

1029-163 Trophic Effect of Angiotensin II in Rat Neonatal Cardiomyocytes are Caused by Endothelin and Non-Myocyte Cells

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Angiotensin II (ANG II) and endothelin (ET) are known to be potent trophic stimuli in various cells including cardiomyocytes. To further characterize these effects we studied, in isolated rat neonatal cardiomyocytes, the effects of the AT_1-receptor (R) antagonist losartan and the ET_A-R antagonist BQ-123 on ANG II- and ET-1-induced inositol phosphate (IP) formation and protein synthesis (as determined by ^{3}H-phenylalanine incorporation). ET's (0.1–1000 nM, ET-1≫ET-3) concentration-dependently increased IP-formation (max. increase: 130% above basal) and protein synthesis (max. increase: 60% above basal). These effects were antagonized by 1 μM BQ-123 but not by 1 μM losartan. Binding studies with [^{125}I]ET-1 revealed a homogeneous class of ET_A-R in the cardiomyocytes. Pretreatment of the cells with pertussis toxin (PTX, 500 ng/ml for 20 h) did not affect IP-formation but reduced protein synthesis by about 40%. ANG II (0.1–1000 nM) increased IP-formation and protein synthesis to a much lesser extent than ET (max. increases: 30% above basal); these ANG II effects were inhibited by 1 μM losartan but also by 1μM BQ-123 indicating that ET-1 may be involved. In well-defined cultures of cardiomyocytes (not contaminated with non-myocyte cells [NMC]) ANG II failed to stimulate IP-formation and protein synthesis while ET's effects were unaltered. Addition of NMC's to the medium restored the ANG II effects. We conclude a) that in rat neonatal cardiomyocytes ET-1 induces protein synthesis by an, at least partly, PTX-sensitive pathway, and b) that the trophic effect of ANG II in rat neonatal cardiomyocytes is brought about via local ET-1 secretion upon AT_1-R stimulation in cardiac NMC's.

1029-164 Magnetic Resonance Imaging Accurately Estimates Cardiac Mass in a Transgenic Mouse Model of Hypertrophy

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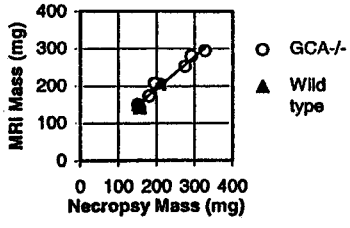
Transgenic mice with a dysfunctional guanylyl cyclase-a gene (GCA–/–) are unable to transduce the signals from atrial naturetic peptide and thus develop hypertension. We used magnetic resonance imaging (MRI) to assess hypertensive cardiac hypertrophy in these animals, using their wild-type sibs as controls. Mice were anesthetized with serial intraperitoneal injections of avertin and gated multislice, multiphase, cine MRI was done at 1.5 T using a conventional imaging system (1.8 mm slices, 196 μ × 250 μ in-plane resolution, field echo sequence). We used Simpson's rule to estimate myocardial mass from 4–5 short axis images. Correlation with heart weight at necropsy was excellent (LV_{MRI} = 0.87 × $LV_{Necropsy}$ +21 mg, r^2 = 0.97). By MRI GCA–/– mass was significantly different when compared to isogenic controls (GCA–/–: 246 ± 42 mg (n = 7) vs. controls: 166 ± 26 mg (n = 3), p < 0.05). Ejection fraction was similar in the two groups (0.72 vs 0.67, p = NS).



In conclusion, MRI allows non-invasive assessment of murine cardiac hypertrophy and will be useful in longitudinal studies of the effects of genetic or pharmacologic manipulation on cardiac hypertrophy and function.

1029-165 Cardiac-Specific Overexpression of Tumor Necrosis Factor-α Causes Lethal Myocarditis in Transgenic Mice

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Tumor necrosis factor (TNF)-α, a proinflammatory cytokine with negative inotropic effects, can be detected in myocardium with end-stage heart failure, after endotoxin administration, and during transplant rejection. Various studies suggest that TNF-α participates in the pathogenesis of cardiac dysfunction. To test this hypothesis, we made a transgenic mouse model which selectively overexpresses TNF-α in cardiomyocytes. A transgene construct was made containing the murine α-myosin heavy chain promoter and the coding sequence of murine TNF-α, followed by the SV40 T antigen intron and polyadenylation signals. Injection of this construct into fertilized eggs yielded 3 transgenic mice, all of which died spontaneously before the completion of weaning. Gross pathological analysis of these mice demonstrated a decrease in body weight with markedly increased heart weight. Histological examination of the heart revealed a substantial, diffuse lymphohistocytic inflammatory infiltrate, associated with interstitial edema. Reverse transcriptase polymerase chain reaction showed that the transgene was expressed in the heart. Enzyme-linked immunosorbent assay demonstrated a substantial amount of TNF-α protein in the transgenic heart. In conclusion, overexpression of TNF-α in the heart leads to severe myocarditis and cardiomegaly. These results support the hypothesis that myocardial expression of TNF-α can contribute to the pathogenesis of cardiac dysfunction.

1029-166 Different Molecular Changes in the β-Adrenergic Signal Transduction Pathway in Primary and Secondary Cardiac Hypertrophy

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In primary cardiac hypertrophy due to hypertrophic obstructive cardiomyopathy (HOCM) the myocardial β-adrenergic signal transduction is desensitized. The aim of this study was to investigate, whether changes in the myocardial β-adrenoceptors (βAR) and GTP-binding proteins are different in primary and secondary myocardial hypertrophy. *Methods:* Hypertrophied septal myocardium was obtained from patients undergoing operation due to HOCM or severe aortic valve stenosis (AoSt). All patients of both groups showed normal left ventricular pump function. Nonfailing myocardial specimens (NF) were from multiorgan donors, whose hearts could not be transplanted. βAR density (Bmax) was measured with ^{125}I-Iodocyanopindolol (ICYP) binding. For βAR-subtype discrimination ICYP was displaced with a β1-adrenoceptor selective antagonist. Determination of alpha subunits of the stimulatory (Gs 52 kDa; 45 kDa not shown) and inhibitory (Gi-2) G proteins was done by immunoblotting with selective antibodies and γ-counting after ^{125}I-Protein A incubation. *Results:* Bmax = maximal ICYP-binding, β1 and beta;2 = βAR-subtypes (in fmol/mg protein); Gs and Gi in 1000 cpm/mg protein. Values are means ± SEM; p < 0.05: $^+$HOCM (n = 9) or AoSt (n = 8) vs NF (n = 4); *AoSt vs HOCM.

	Bmax	β1	β2	Gs (52 kDa)	Gi-2
HOCM	44 ± 4$^+$	24 ± 3$^+$	20 ± 2	11 ± 1	9 ± 1*
AoSt	38 ± 4$^+$	24 ± 3$^+$	15 ± 2	16 ± 2^{+*}	6 ± 0.5
NF	70 ± 7	51 ± 6	19 ± 2	10 ± 1	6 ± 1

β1-AR were selectively down-regulated in HOCM as well as in AoSt. Whereas in HOCM the inhibitory Gi-2 was increased, in AoSt the stimulatory Gs was increased. *Conclusions:* In HOCM myocardial βAR and G proteins are changed towards desensitization of the β-adrenergic signal transduction pathway whereas they are changed counteractively in secondary myocardial hypertrophy due to AoSt. The changes in the β-adrenergic signal transduction pathway in hypertrophied human myocardium depend upon the etiology of the hypertrophy.

1030 Neural Control of Cardiac Function in Hypertension

Tuesday, March 18, 1997, 3:00 p.m.–5:00 p.m.
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1030-45 Relationship of QT Dispersion to LVH in Patients With Hypertension

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In patients with hypertension, LVH is associated with an increased risk of sudden cardiac death. The mechanism is incompletely understood. Dispersion of the QT interval is an index of repolarization inhomogeneity, and increased QT dispersion may indicate enhanced susceptibility to ventricular arrhythmias.

To determine the relationship between QT dispersion and LV mass, we